PARTICULATE MATTER

Peer Review
Experimental Toxicology Division
N H E E R L

Comparative Genomic and Proteomic Approaches to Determine Molecular Mechanisms of Particulate Air Pollution Cardiopulmonary Toxicity

Srikanth S Nadadur, Kevin L. Dreher, and Daniel L. Costa, US EPA, NHEERL, ETD, PTB

Introduction

- Epidemiological observations have associated adverse health effects to ambient PM exposure despite regional differences in their composition.
- The complex and dynamic nature of ambient air PM as well as population demographics adds a unique dimension to the complexity of our efforts to identify the biological basis for PM: hazard identification, mechanism of injury and susceptibility factors.
- High throughput comprehensive toxicological screening technologies and bioinformatics are needed in order to identify: 1) molecular response profiles that can be used as biomarkers of exposure and toxicity; 2) target and effector organs and cell types; 3) specific causal constituents within ambient air and emission source PM; and 4) mechanisms of injury and susceptibility.
- Initial efforts towards identification of target cell and PM causal constituents PM involve an integrated approach of correlating transcription factor activation/inactivation, gene, and protein expression profiles using microarray technologies.
- Computational models of cellular and/or tissue molecular responses can be developed in order to effectively extrapolate between animal and human PM *in vitro* and *in vivo* studies involving validated bio-response signature(s) of adverse PM health effects.

Methods

Genomic Studies:

Cells: Primary human vascular endothelial cells (HUVEC) and rat pulmonary vascular endothelial cells (RLVEC) were purchased from VEC Technologies (New York, NY).

***In Vitro* Exposure:** Acute (25 min) exposure of HUVEC and RLVEC to: 1) ROFA 1μg/ml (a residual oil fly ash rich in V as well as Ni & Fe); 2) Vanadium sulfate (1 μM); or 3) Saline.

Gene expression analysis: Atlas human 8K and rat 4k plastic microarray (Clontech, Palo Alto, CA).

Data Analysis: Atlas Image Analysis 2.7 (Clontech, Palo Alto, CA), GeneSpring software (Silicon Genetics, Redwood City, CA).

Proteomic Studies:

Combustion PM: PM<2.5 particles were collected from the combustion of residual oil, coal, and truck diesel fuels.

***In Vivo* PM Exposure:** Rats were exposed to saline or various combustion PMs by intratracheal instillation (1 mg/rat). Samples were divided into a training (25 rats) and testing (25 rat) set for each exposure group at each post-exposure time point.

Proteomic Analysis: Plasma was recovered from saline or combustion PM exposed rat at 2 – 3h and 24h post-exposure. Proteomic analysis was performed on plasma samples using a using the IMAC and WCX2 Chipergen protein chips. SELDI-TOF spectra derived from each protein chip were analyzed using a pattern recognition bioinformatic software designed to identify unique MS signature profiles.

Genomics

Fig.1. O'mics Based Computational Models

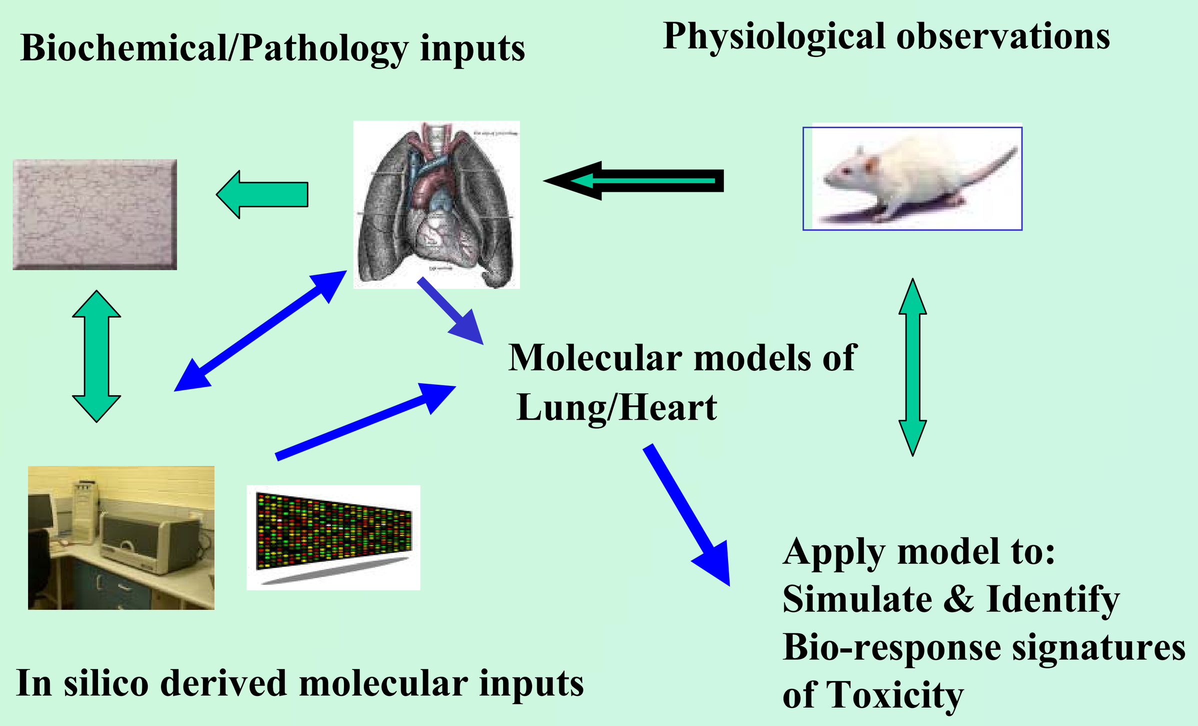

Fig. 2. Scatter plot showing differential gene expression profiles in HUVEC on acute exposure to 1μg ROFA and 1 μ M Vanadium

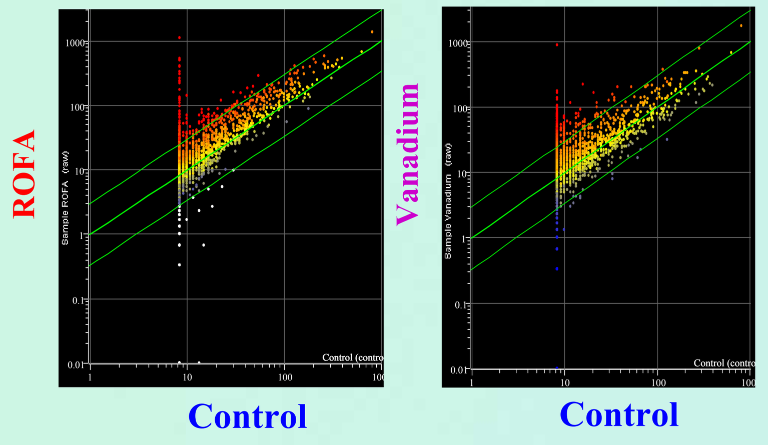

Fig.3. Classification of genes found induced by 3-fold in HUVEC and RLMVEC into three functional groups that are unique to ROFA, V and common.

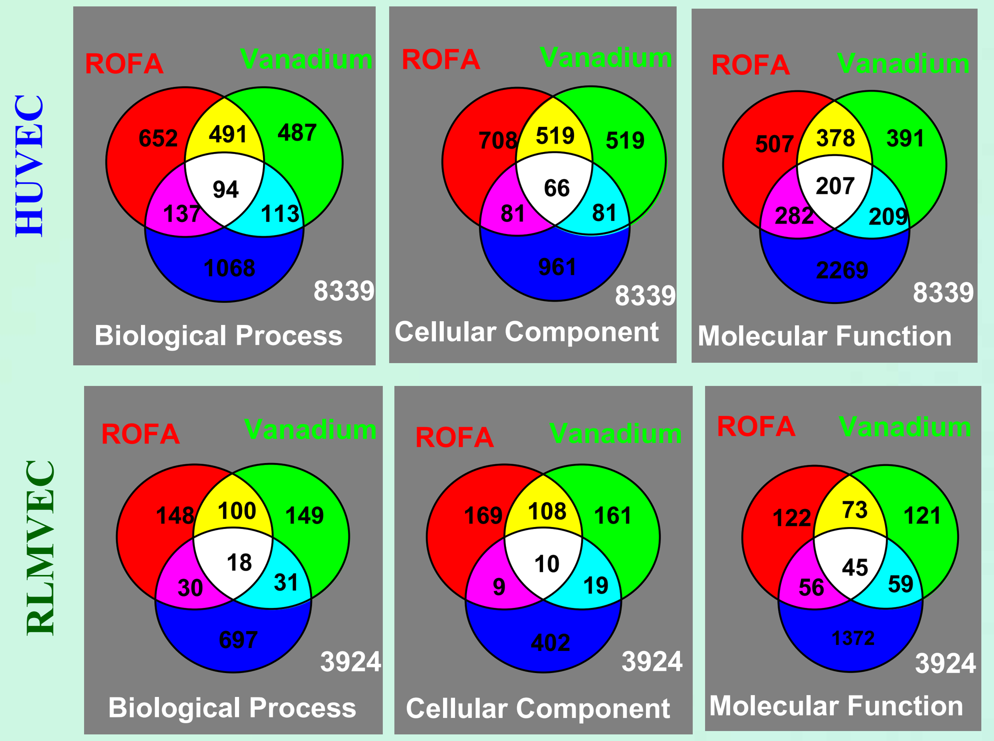

Table1. Differential expression in three gene families that are common and unique to ROFA and Vanadium

Group	ROFA		Vanadium		Common	
	Up	Down	Up	Down	Up	Down
Interleukins (39)	2	8	2	5	4	2
Extracellular Matrix proteins (126)	1	13	2	4	13	0
Growth Factors, Cytokines & Chomokines (189)	14	1	14	3	3	10

Table 2. Differential expression in three gene families that are common and unique to ROFA and Vanadium in RLMVEC

Group	ROFA	Vanadium	Common
Interleukins (8)	0	1	0
Extracellular Matrix proteins (10)	0	2	1
Growth Factors Cytokines & Chomokines (33)	1	3	2

Proteomics

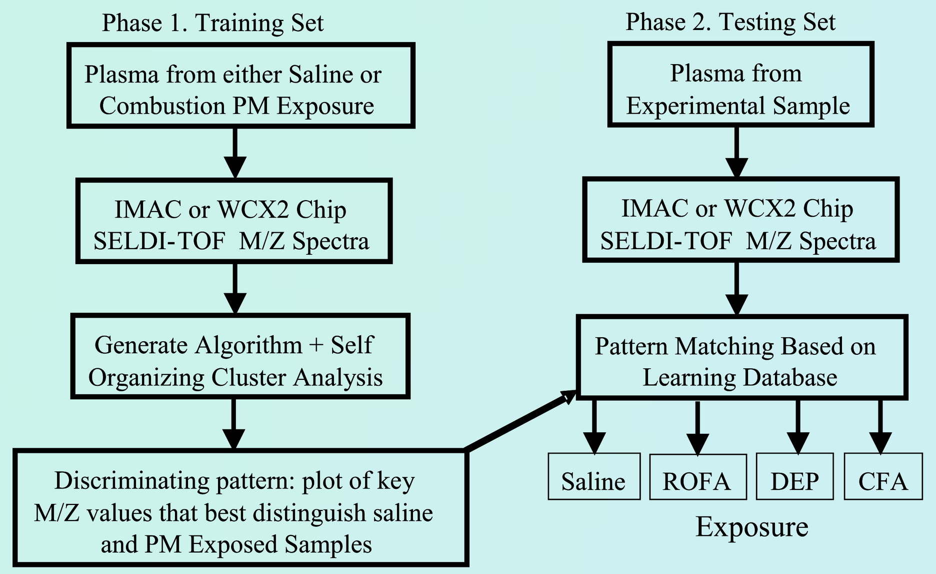

Fig.4. Phases of N-dimensional Proteomic Analysis to Identify Biomarkers of Exposure and Toxicity. Plasma will be recovered from rats exposed to either saline or various combustion particles and analyzed by Ciphergen protein chip analysis as outlined above in order to identify plasma protein profiles associated with combustion PM exposure and\or toxicity.

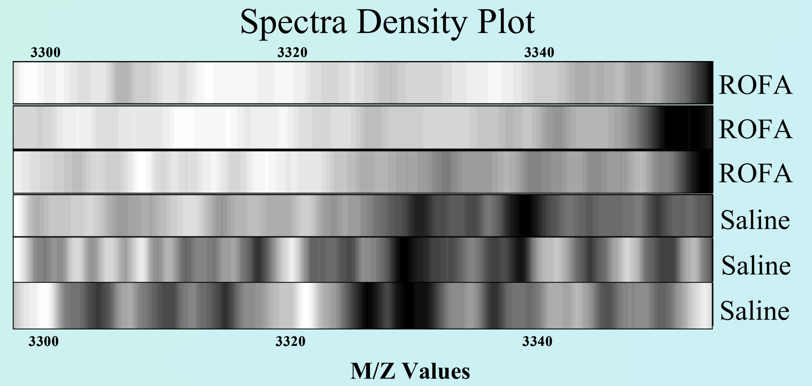

Fig.5. Preliminary data showing representative SELDI-TOF mass spectra density plots of plasma recovered from saline or ROFA exposed rats at 24h post-exposure.

Table.3. Preliminary Proteomic Analysis of ROFA Exposed Rats

Time Post-Exposure	# and Type of Training Samples	# and Type of Test Samples	% Sensitivity (Blinded Test Set)	% Specificity (Blinded Test Set)
2 – 3 h	9 ROFA; 13 Saline	13 ROFA; 11 Saline	89	100
24 h	11 ROFA; 10 Saline	13 ROFA; 14 Saline	100	100
All	20 ROFA; 23 Saline	26 ROFA; 25 Saline	62	100

Initial results to test the proof of concept that one would be able to employ plasma proteomic analysis and pattern recognition bioinformatics software are extremely encouraging. As shown in the above Table only a small number of training plasma samples were needed for saline and ROFA exposure to correctly predict the correct exposure group of all the test samples.

Conclusions

Genomics: Gene expression profiles derived from HUVEC & RLVEC exposed to similar concentration of ROFA and V indicated species specificity in PM toxicity. Although there was a significant difference in the number of genes altered between human and rat cells. Identification of certain common genes (TGF-β, TNF-R and MAP K1) suggest that acute injury response mediation may involve similar genes in other types of cells and in vivo.

Proteomics: Plasma proteomic profiling may provide an approach to identify systemic bio-response signature(s) of exposure and/or toxicity following exposure to various types of combustion particles.

Impact

Comparative cell and tissue specific genomic and proteomic databases developed integrating *in vitro* and *in vivo* studies with classical toxicological investigations will aid in developing better predictive models for ambient PM bio-response signature(s) identification for risk assessment paradigms.

Future Directions

An integrated genomic – proteomic approach to link cell signaling pathways with gene expression in human and rat cardiopulmonary cells or tissues will allow for a systems biology approach to develop computational models from which bio-response signature(s) of PM toxicity and/or exposure to be derived.

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